DOCUMENT RESUME

ED 091 233 SE 017 817

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TITLE Plant Hormones: How They Affect Root Formation.
INSTITUTION Agricultural Research Center (DOA), Beltsville, Md.
PUB DATE Apr 74
NOTE 11p.; Science Study Aid No. 9
AVAILABLE FROM Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 ($0.10)

EDRS PRICE MF-$0.75 HC-$1.50 PLUS POSTAGE
DESCRIPTORS Biology; *Botany; Instruction; *Instructional Materials; Learning Activities; Plant Growth; *Plant Science; Science Education; *Secondary School Science

ABSTRACT

This science study aid, produced by the U.S. Department of Agriculture, includes a series of plant rooting activities for secondary science classes. The material in the pamphlet is written for students and includes background information on plant hormones, a vocabulary list, and five learning activities. Objectives, needed materials, and procedures are specified for each of the activities. Questions to be answered by the students are included when appropriate. Supplementary activities are suggested. (DT)

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SE 017 817

PLANT HORMONES

How they affect root formation

U.S. DEPARTMENT OF AGRICULTURE

Science Study Aid No. 9

TEACHER'S INTRODUCTION

This Science Study Aid includes a series of plant rooting activities for secondary science classes. The activities demonstrate that plant growth, shown by the first stages of rooting, usually occurs in an organized and predictable manner and is influenced by the balance of naturally occurring chemicals known as plant growth regulators and hormones.

These activities are an aid to studying plant growth. Students should have some knowledge of basic stem and root structure. The activities can be conducted indoors at any season of the year.

Mung, kidney, or pinto beans are suggested as test plants, because they germinate quickly, grow rapidly, and are hardy. Bean seeds and plants can be purchased from seed stores and organic food stores.

Vermiculite, used here as a growth medium, can be reused once the old roots and stem stumps have been removed.

Activity I, Growing Beans From Seed, can be conducted as either a class activity or completed by the teacher 5 to 7 days before seedlings are needed for Activities II through V.

The material that follows this Teacher's Introduction is set up for easy reproduction. It is written for students. You may wish to reproduce and distribute it all at once, or section by section, as you take up each activity.

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Plant Hormones: How They Affect Root Formation was developed by Mrs. Diana Hereda Reinhard, a high school biology teacher in Baltimore County, Maryland, with the cooperation of Dr. Luis E. Gregory and Dr. Joseph F. Worley of the Plant Hormone and Regulator Laboratory, Agricultural Research Service, Beltsville, Maryland.

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BACKGROUND INFORMATION

Stem *cuttings** are taken from the parent plant and include a segment of stem, usually with several leaves attached. Some scientists believe the leaves provide nutrients and are a source of *plant hormones*, which induce root formation.

The relationship of these chemicals strongly influences plant development. Plant hormones are formed in one tissue or organ of the plant and are *translocated* to other sites. Very small quantities of these hormones cause marked effects on plant growth.

These hormones, also known as *phytohormones*, can promote, *inhibit*, or otherwise modify *physiological* processes in plants. The multiple physiological functions of hormones often overlap. In a given plant process, the action of one hormone may be similar to or opposed to another. Hormone action is sometimes *synergistic*. In other cases, one hormone may act independently of other hormones in the plant.

Plant growth regulators, which include hormones as well as synthetic materials that produce hormone-like effects, may stimulate or retard growth of plants. Plant response depends on the kind and concentration of the chemical, the length of time the tissue is exposed, and the age of the plant. There are many known plant regulating chemicals, but one of the most widely studied is *indoleacetic acid*, a naturally occurring hormone.

Indoleacetic acid and certain other naturally occurring and synthetic chemicals with similar properties are known as *auxins*. They produce elongation and division of stem cells, stimulate flower formation in some plants, affect *cambial* activity, and stimulate *adventitious* root formation.

Scientists do not know exactly how auxins stimulate root formation. However, researchers at USDA's Agricultural Research Service (ARS) in Beltsville, Md., have found that roots of mung beans and pinto beans form an unknown substance that inhibits the formation of further roots on cuttings after a certain number of roots have been produced. The extent of this limited root growth can be predicted from observation of the internal or external structure of the plants, as the following activities show.

Applications of auxins usually accelerate root formation and thus reduce the time required for rooting. Applying auxins does not cause something unnatural to occur but rather speeds up a process that would, in time, have occurred naturally. There are, however, a few differences between naturally produced roots and those induced by application of auxins. Naturally occurring roots are fewer in number and longer, roots induced by auxins are more numerous but shorter, making the plants better suited for transplanting. The effectiveness of auxins in root initiation has led to their widespread use in *asexual propagation* of plants.

The use of synthetic hormones has contributed significantly to American agriculture, improving food quality as well as increasing quantity. For example, growth regulating chemicals are used to hold fruit on the trees until they can be picked, so they are not damaged by hitting the ground. Sprouts on potatoes used to be a serious storage problem until a chemical to inhibit sprouting was developed by the late Dr. Paul C. Marth of the Agricultural Research Service. Thompson seedless grapes are commonly treated with gibberellic acid to increase the size of the grapes and open up the clusters. This allows better air circulation and reduces disease problems.

Researchers have found, however, that some synthetic hormones are harmful to people and other elements of the environment. Because natural homones have not produced any serious ecological problems, agricultural researchers at the ARS Plant Hormone Regulator Laboratory in Beltsville are searching for natural hormones that will control plant growth and development efficiently without danger to consumers or to the environment.

If you find the following activities interesting and wish to go on to other related projects, an excellent reference is *Methods of Studying Plant Hormones and Growth Regulating Substances* AH-336. It is available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

*Italicized words are defined in the vocabulary (p. 3)

VOCABULARY

Adventitious - roots that arise from mature tissues or from parts of the plant where roots would not arise under ordinary conditions of growth.

Apical bud - terminal growing point of the stem.

Asexual propagation - reproduction of plants using vegetative parts such as leaves or stems. (See *cutting* listed below.)

Auxins - plant regulating compounds characterized by their capacity to induce cell division and cell growth.

Bromthymol blue - a blue dye used to stain plant cells for observation.

Cambial - cells which give rise to new cells and are responsible for lateral or secondary growth.

Cotyledons - in seed plants, the first leaf or leaves developed within the embryo in the seed.

Cotyledon scars - marks left on the stem when the cotyledons fall off.

Cutting - section of a plant which can grow into a new plant. (See *asexual propagation*.)

Dicotyledon, or dicot - a seed plant with two cotyledons.

Epicotyl - portion of the stem above the cotyledons.

Germination - in seed, the period from the end of dormancy to independent food-making activity of the plant.

Hypocotyl - portion of the stem between the cotyledons and the roots.

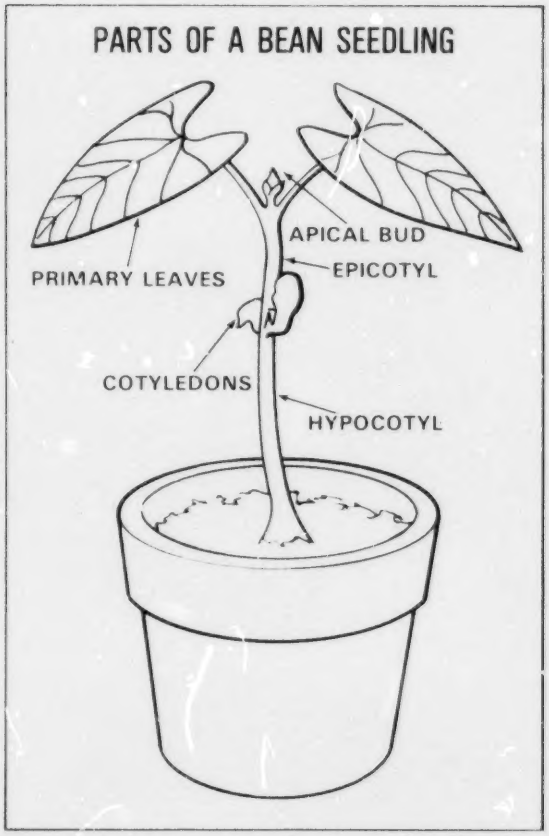
Hypothesis - a tentative assumption to be tested with experiments and observations.

Indoleacetic acid (IAA) - a natural plant-growth hormone.

Phototropic reaction, or phototropism - the response of a plant to light—the plant or its parts bend toward the light.

Physiological - functions of the organs of plants and animals.

Plant hormones, or phytohormones - chemical regulators, produced by plants, which in low concentrations regulate plant physiological processes. They are produced in one area of a plant and translocated to other areas where the response occurs.



Plant growth regulators - organic compounds, other than nutrients, which in small amounts promote, inhibit, or otherwise modify any physiological process in plants. (Note: plant growth regulators include both synthetic and natural materials. Plant hormone refers only to the natural hormones produced by the plant.)

Primary leaves - first true leaves of a plant.

Primordium - beginning of development of an organ. Adj. - primordial.

Seedling - a plant, still immature, grown from a seed.

Synergistic - acting in cooperation, working together, mutually reinforcing.

Translocation - transfer of nutrients or products of metabolism from one location to another in the body of a plant.

Vascular bundles - the strands of cells of the vascular system which conduct substances throughout higher plants.

ACTIVITY I: Growing Beans From Seed

Objective: To observe the germination and growth of a *dicotyledonous* plant, and to provide bean seedlings for a series of experiments on root emergence.

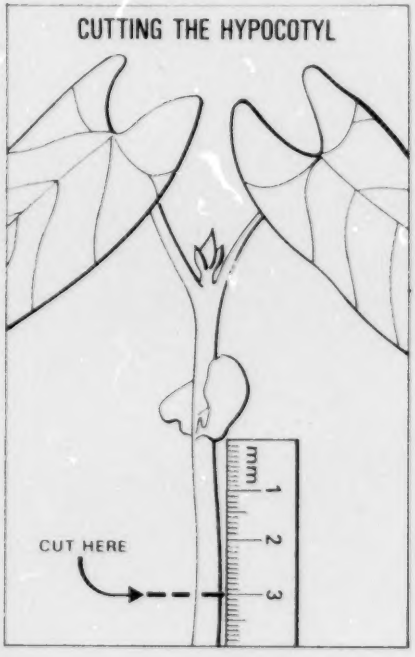
Materials: Bean seedlings (mung beans, pinto beans, or kidney beans), approximately 30 ml. of mung bean seeds will yield about 300 usable seedlings; 160 ml. of pinto or kidney beans yield about 300 usable seedlings.
Vermiculite or other artificial soil mix, approximately 4 quarts.
Planting tray, approximately 22 x 11 inches.
Graduated cylinder, 100 ml.
Clorox (5.25% sodium hypochlorite), 5 ml.
Tap water, approximately 1 liter.
Light source, such as a window.

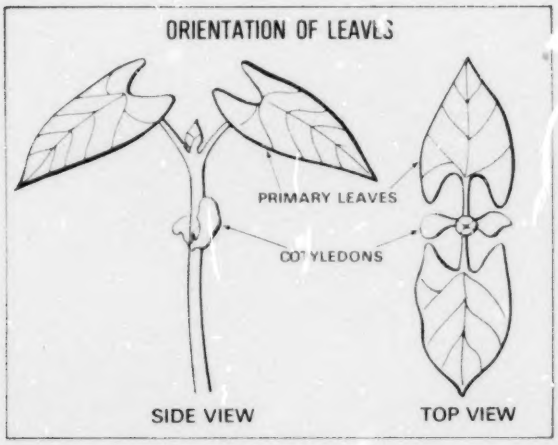
Procedure:
1. Disinfect seeds.
   a. Soak for three minutes in a solution of 5 ml. of Clorox in 70 ml. of water.
   b. Rinse the seeds thoroughly with running water.
2. Start the first step of the *germination* process. Soak disinfected seeds in water overnight.
3. Place vermiculite in the planting tray and moisten with tap water. The vermiculite should be damp to your touch. Drain excess water from the tray.
4. After the seeds have absorbed water, they should be planted ¼ to ½ inch deep in the vermiculite.
5. For the next 5 to 7 days—
   a. If the light comes from one side, rotate planting tray daily to balance out the plants' *phototropic reaction*.
   b. Add water only when the vermiculite looks or feels dry.
6. When the bean seedlings have their two *cotyledons* and two *primary leaves* opened, you are ready for ACTIVITY II.

ACTIVITY II: Predicting Root Emergence From an External View of Cuttings

Objective: To verify predictions of where roots will emerge by observing the stem locations of cotyledons and primary leaves.

Materials: Bean seedlings from Activity I, six per small group of students.
Scalpel, one per group.
India ink and pen, one set per group.
Metric ruler, one per group.
Small, narrow container, vial, medicine bottle or 50 ml. flask, one per group.





Procedure:

1. Select six seedlings with two cotyledons and primary leaves (usually two.) At this stage the seedlings are about seven days old. Work with one seedling at a time.
2. Using a scalpel, cut off seedlings at soil line, leaving roots in the growing medium.
3. To insure that the *hypocotyls* of all selected seedlings are as uniform as possible, place each seedling along the edge of a centimeter ruler. Measure 3 centimeters down from the cotyledons or (if the cotyledons already have fallen off) measure from the *cotyledon scars*.
4. Cut each hypocotyl so that it is just 3 centimeters long below the cotyledons. See drawing on p. 4.
5. Draw an india-ink vertical line along the stems from each of the two cotyledons (cotyledon scars) from each of the two primary leaves to the base of the hypocotyl. These lines represent your prediction of where roots will emerge from the cuttings, based on the *hypothesis* that roots emerging from the stem will be aligned with the cotyledons and primary leaves.
6. Allow the ink to dry for 2 minutes.
7. Group the cuttings, according to their maturity, and place them in small containers with 2 cm. of tap water. Use no more than six cuttings in a container.
8. Maintain the 2 cm. water level for the next 7 days. During the period, observe whether roots emerge along the inked lines.
9. Do not throw away the water left in the container. It will be used in ACTIVITY IV

Further Activities and Questions:

1. Keep a daily log on each of your cuttings for 7 days. Include:
   a. Days on which the roots emerge.
   b. Locations of the roots in relation to prediction lines.
   c. Number of roots that emerge.
2. Was your ink-line prediction of where the new roots would emerge correct? What is the relationship, if any, between the position of the primary leaves and cotyledons and the location of new root formation?
3. Is there any relationship between the state of activity of the *apical bud*, or the presence or absence of cotyledons, and the number of new roots? If there is a relationship, what is it?
4. If you get positive results with this experiment, you may wish to know if the same relationships are found in other plants. Scientists have not as yet tested this hypothesis on a wide variety of species, so this is an area in which you can do original research.

5. To see what happens when you cut the stem at a different place, cut some seedlings through the *epicotyl* about halfway between the primary leaves and the cotyledon. Place the cuttings in water for five to seven days and then compare the root emergence pattern to that of the hypocotyl cuttings. Describe your findings.

ACTIVITY III: Predicting Root Emergence From an Internal View of Cutting Cross Sections

Objective: To observe the relationships between the orientation of *vascular bundles* in plant stems and the emergence of roots.

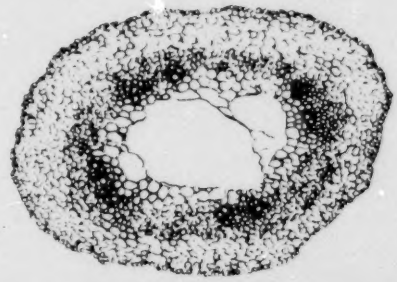
Background: When roots emerge, the first recognizable structure is an organized root *primordium* within the stem. It is usually found toward the outside of a vascular bundle. After its initiation, the primordium grows into a root, and this projects through the tissues of the stem as the visible root.

Materials: Beans (mung, kidney, or pinto) with 3 cm. hypocotyl from ACTIVITY I or II, 6 per group.
Bromthymol blue, approximately 10 ml. per group (mix 0.5 g. bromthymol in 500 ml. distilled H_2O and add one drop of ammonium hydroxide).
Small container, such as a watch glass, 1 per group.
Grease pencil or slide labels.
Scalpel, 1 per group.
Microscope.
Microscope slides, 6 per group.

Optional: Cover slips, 6 per group.
Fingernail polish, clear, 1 bottle to be shared by all groups.
Glass rod or eye dropper, 1 per group.
Glycerin, approximately 10 ml. per group.
Probe, 1 per group.

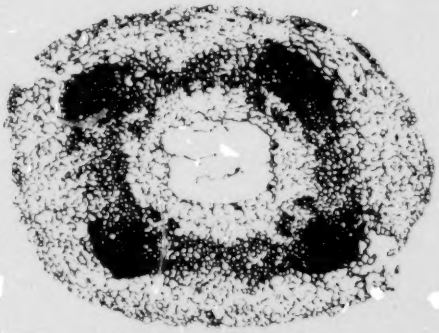
SECTIONS OF A HYPOCOTYL

A



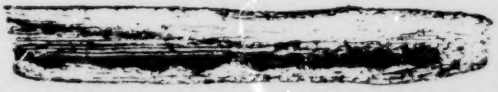
Cross section immediately after cutting. Note four groups of large, dark, xylem bundles.

B



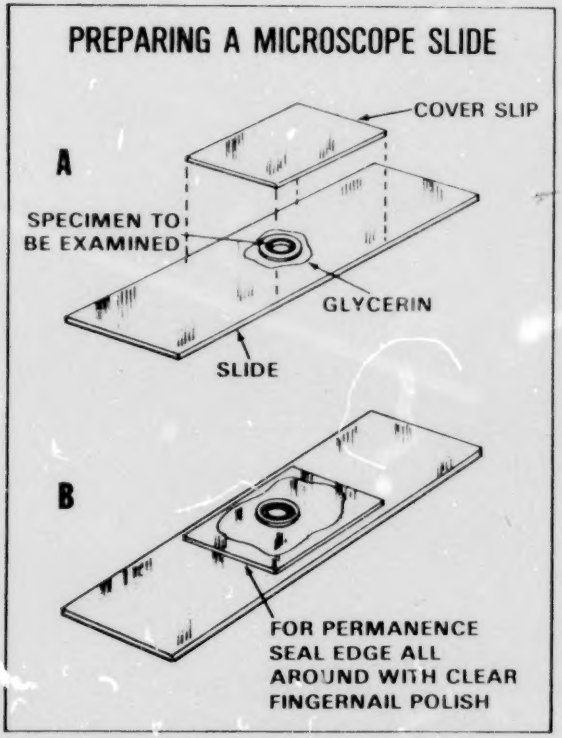
Cross section of 4-day-old cutting. Four root primordia are formed near the 4 groups of xylem bundles shown in A.

C



Longitudinal section of 4-day-old cutting showing placement of primordia along hypocotyl.

Procedure:
1. Prepare six seedlings as shown in steps 1 through 7 of ACTIVITY II.
2. Place the six cuttings in a small container with 2 cm. of tap water. Maintain the 2 cm. water level for the next 5 days.
3. Beginning with the day of initial cutting and continuing for the next 5 days, work with one cutting each day.
   a. Place about 2 ml. of bromthymol blue solution in a container.



b. Put the base of the cutting in the bromthymol blue.
c. Allow the hypocotyl of the selected cutting to soak in the bromthymol for 1 minute, until it is lightly stained.
d. Rinse the hypocotyl in running tap water to remove excess blue stain.
e. Drain the hypocotyl and dry gently, blotting it between paper toweling.
f. Place the hypocotyl on a glass plate or slide. Using a scalpel, cut very thin, even cross sections of the hypocotyl.
g. Place these sections on clean microscope slides and observe them under the microscope, using both low and high power.

4. Sketch a microscopic cross-section view of each day's growth. Observations should be concentrated on the vascular-bundle area of each cross section.

Questions:

1. How many vascular bundles did you observe?
2. Are the vascular bundles grouped in any particular manner? If so how?
3. In what day of growth did you begin to see evidence of root primordium in the stem?
4. In what day of growth did you see evidence of roots beginning to emerge from the stem?
5. Is there a relationship between the location of the vascular bundles and the location of root emergence? If so, what is that relationship?

SUPPLEMENTARY ACTIVITIES

1. If you find some especially good cross-section views, you may wish to keep them for future reference. Preserve the cross section as follows:
   a. Place one or two drops of glycerin on a microscope slide with a glass rod or eye dropper.
   b. Place the selected hypocotyl cross section in the glycerin and add additional glycerin until it covers the cross section in an even layer.
   c. Hold a cover slip horizontally over the glycerin area, and then let it drop gently onto the glycerin.
   d. Allow the cover slip to settle for 15 to 30 minutes, then seal the edges of the cover slip and slide with clear fingernail polish.

e. Label the slide with the day of growth.
f. Allow the prepared slide to dry on a flat surface for about 3 days.

2. Make a cross section of the epicotyl region, using the procedures described in steps 3 and 4 of ACTIVITY III.
   a. Sketch the microscopic view of this section
   b. What differences do you observe between the epicotyl and hypocotyl regions?

ACTIVITY IV: How Plant Extracts Affect Rooting

Objective: To observe whether initiation of roots on bean cuttings can be increased by soaking the cuttings in washings from other roots.

Materials: Bean seedlings (mung, kidney, or pinto), 8 per group.
Containers, small, such as vials, medicine bottles, or 50 ml. flasks, 2 per group.
Scalpel, 1 per group.
Water from earlier bean experiments (See ACTIVITY II, Procedure 9).

Procedure:
1. Collect the water from the small containers of seedlings that you used in ACTIVITY II.
2. Place 2 cm. of the water in one small container, labeled "old water." (Use a cm. ruler to measure this height.)
3. Place 2 cm. of fresh tap water in the remaining container, labeled "fresh water."
4. Using a scalpel, cut the stems of eight seedlings, 3 cm. below the cotyledons.
5. Place four cuttings in the container of "old water" and four additional cuttings in the container of "fresh water."
6. For the next five to seven days, observe and compare the growth rates of roots in the two containers.
7. Keep a daily log of your observations. Include:
   a. When the new roots emerge.
   b. Where the new roots emerge.
   c. The number of roots that emerge.

Questions:
1. Is there a difference in the rate of development of the roots in the two containers? If so, what is the difference?
2. Is there a difference between the number of roots that emerge in the two containers? If so, what is the difference and to what factors can you attribute it? (You may deduce that hormones in the "old water" affected the rooting of cuttings soaked in this water. How could the observed differences in root growth be specifically related to hormones in the "old water"?)

SUPPLEMENTARY ACTIVITIES

Objective: To observe whether production of roots can be increased by treating cuttings with synthetic hormones.

Materials: Bean seedlings (mung, kidney, or pinto), 16 per group.
Containers, small (medicine bottles or vials), 5 per group.
Scalpel, 1 per group.
Flask, volumetric, 100 ml., 4 per group.
Indoleacetic acid (IAA), or commercial preparation containing IAA, such as Rootone, 140 ml.

Procedure:
1. To obtain maximum effects, prepare the following concentrations of indoleacetic acid (IAA):
   a. Weigh out 8.0 mg. of indoleacetic acid; place this amount in a 100 ml. flask and fill with water to the 100 ml. line. This will give an 80 mg/liter concentration.

b. Weigh out 6.0 mg. of indoleacetic acid; place this amount in a 100 ml. flask and fill with water to the 100 ml. line. This will give a 60 mg/liter concentration.

c. In a 100 ml. volumetric flask, pour 50 ml. of 80 mg/liter concentration and add water to the 100 ml. line. This will give a 40 mg/liter concentration.

d. In a 100 ml. volumetric flask, pour 50 ml. of the 40 mg/liter concentration and add water to the 100 ml. line. This will give a 20 mg/liter concentration.

2. Label each of the five containers, 80 mg/liter IAA, 60 mg liter IAA, 40 mg/liter IAA, 20 mg/liter IAA, and control.
3. Into each of your four test containers, pour 2 cm. of one of the prepared concentrations of indoleacetic acid. As a control, place 2 cm. of plain tap water into the fifth container.
4. Using a scalpel, cut the stems of 20 seedlings, 3 cm. below the cotyledons.
5. Place four of the cuttings in each of the 4 concentrations and in the control.
6. For the next 5 to 7 days, maintain the 2 cm. water level. Observe and compare the growth rates of the roots in the varying concentrations of indoleacetic acid.
7. Record your observations in a daily log. Include:
   a. When the new roots emerge.
   b. Where the new roots emerge.
   c. The number of roots that emerge.
8. Compare the growth rates of the roots in the varying concentrations of indoleacetic acid with the growth rates of roots grown in plain tap water and in water from older cuttings.

ACTIVITY V: How Presence or Absence of Roots Affects Emergence of Other Roots

Objective: To observe how emerged roots inhibit the growth of additional roots, and how severing the hypocotyl section containing emerged roots stimulates the growth of additional roots on the remaining hypocotyl.

Background: Permit the bean seedlings to grow until four rows of secondary roots are evident. At this point something inhibits further root formation in the cutting. However, if the section of the stem which contains the roots is removed, this inhibitor is no longer evident. Therefore, new roots are able to form at a higher point on the stem. This can be observed in the following activity.

Materials: Bean seedlings (mung, pinto or kidney) with their 4 rows of roots intact, 8 per group.
Containers, small, such as vials, medicine bottles, or 50 ml. flasks, 2 per group.
Sharp scalpel, 1 per group.
Tap water, 4 cm.

Procedure:
1. Select eight seedlings that have four rows of roots intact.
2. Split the hypocotyl of all seedlings vertically for 2 cm.
3. Place four split-stem seedlings in a small container with approximately 4 cm. of tap water.
4. Take the four remaining seedlings and cut off one of the sides of the split stem at the uppermost point of the cut.

5. Place the four stem cuttings in another container with approximately 4 cm. of tap water.
6. For the next 5 to 7 days observe and keep a log on the split-stem cuttings. Record:
   a. When the new roots emerge.
   b. Where the new roots emerge.
   c. The number of roots that emerge.
7. Using data from the log:
   a. Compare root development in the two types of split stems.
   b. What similarities do you observe in the two types of split stems?
   c. What differences do you observe in the two types of split stems?
   d. To what do you attribute these similarities and differences?
   e. What would happen if the rooted stem was not split but some of the roots were simply "shaved" off?

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